

~~MRID 00118408~~ 7  
MRID 00118048 FORM I

TDMS

DATA EVALUATION RECORD

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CASE GS 0229

Norflurazon

PM 06/04/84

CHEM 105801

Norflurazon (4-chloro-5(methylamino)-

BRANCH EEB

DISC       

FORMULATION 96.6% A.I.

FICHE/MASTER ID FAONOR04

CITATION: Early-life stage toxicity study in the rainbow trout on Norflurazon  
conducted at EG&G Bionomics (BW-82-5-1165 Sandoz Project  
T-1733) Acc. #248839 Dated 10/18/83  
248829

SUBST. CLASS= S

OTHER SUBJECT DESCRIPTORS  
PRIM:

DIRECT REVIEW TIME= 2.0 (MH) START DATE 5/30/84 END DATE 6/7/84

REVIEWED BY: Russel Farringer, III  
TITLE: Wildlife Biologist  
ORG: EEB/HED  
LOC./TEL: 557-7560

SIGNATURE: *Russel Farringer*

DATE: 6/7/84

APPROVED BY:

TITLE:

ORG:

LOC./TEL:

SIGNATURE:

DATE:

This study was scientifically sound. The MATC of rainbow trout continuously exposed to a range of Norflurazon concentrations (0.77, 1.5, 3.3, 5.5, 11.2) during embryo to larvae stage is >0.77 mg/l <1.5 mg/l (based upon survival and growth of larva). This study fulfills the requirement for the aquatic fish early life cycle study.



*Duplicate  
sent*

DATA EVALUATION RECORD

1. CHEMICAL: Norflurazon (EPA Reg. No. 11273-13,19)
2. FORMULATION: 96.6% A.I.
3. CITATION: Early-life stage toxicity study in the rainbow trout on Norflurazon conducted at EG&G Bionomics (#BW-82-5-1165 Sandoz Project T-1733) Acc.# 248839 Dated 10/18/82
4. REVIEWED BY: Russel Farringer, Wildlife Biologist  
Ecological Effects Branch  
Hazard Evaluation Division (TS-769)
5. DATE REVIEWED: January 4, 1983
6. TEST TYPE: Fish Early-life Stage  
  
TEST SPECIES: Rainbow Trout
7. REPORTED RESULTS: Percent viability was not statistically significant ( $p < 0.05$ ) between treatment and control groups. No toxicological signs were observed at concentration 0.77 mg/l. The MATC of Rainbow trout exposed to Norflurazon during embryo to larvae stage is  $>0.77$  mg/l.  $<1.5$  mg/l.
8. REVIEWER'S CONCLUSIONS: This study was scientifically sound. The MATC of Rainbow trout continuously exposed to a range of Norflurazon concentrations (0.77, 1.5, 3.3, 5.5, 11.2) during embryo to larvae stage is  $>0.77$  mg/l  $<1.5$  mg/l.

## MATERIALS AND METHODS

### TEST PROCEDURES

This study generally follows the EPA guideline requirements of July 10, 1978 and protocols that were delineated in that document in regards to flow-through aquatic testing with fish. The nominal test levels were determined to be present at or above the stated level by GLC-ECD. The embryo and larvae exposure was initiated within 2 hours after egg fertilization and continued through 30 days post swim-up. Fifty embryos were used in each test chamber with two test chambers power control, solvent control and nominal concentration were utilized. An unknown number of larvae were utilized in replicate tanks (2) for each test level. Larvae were fed brine shrimp (*Artemia salina* nauplii) three times daily on weekdays and twice daily on weekends and holidays. Aquaria were brushed and siphoned at least twice each week or as necessary to remove excess food and fecal matter. Solvent controls (dimethyl formamide at 100 microliters/L) were utilized at the highest solvent/ toxicant dosing. DO, pH, and temperature were measured daily in one set of replicate tanks with all tanks being measured at least once a week. All test solutions were sampled on test day 0, day of hatch and weekly thereafter to assure that the nominal concentration and actual concentration were relatively the same. A stock sample was collected from the primary holding tank at each sample interval.

### STATISTICS

Percentage hatch of embryos and survival, length and weight of larvae after 30 days exposure, were subjected to analysis of variance (Steel and Torrie, 1960, completely randomized block design,  $P=0.05$ ). Data for percentage hatch and percentage survival were transformed to  $\arcsin(\sqrt{\text{percentage}})$  prior to analysis. If treatment effects were indicated, the means of these parameters were compared to those from the control and solvent control using Dunnett's procedure (Steel and Torrie, 1960). When a treatment mean was significantly different from the control means ( $P=0.05$ ), that treatment was considered to be an effect level. Based on these data, the MATC of Norflurazon to fathead minnow embryos and larvae was estimated.

### RESULTS

The Maximum Acceptable Toxicant Concentration (MATC) of Rainbow Trout when continuously exposed to Norflurazon during the embryo to larvae stage is  $>0.77$  mg/l  $<1.5$  mg/l.

### REVIEWER'S EVALUATION

#### TEST PROCEDURE

This study appears to have been conducted following guideline requirements for an embryo to larvae study.

## STATISTICAL RESULTS

ANOVA analysis was performed on this data set. The independent variable was treatment level, dependent variables were percentage of hatch and survival, length and weight.

## S T A T I S T I C A L   A N A L Y S I S   S Y S T E M

OBS	TREAT	HAT	SUR	LEN	WT
1	C	26	88	80	46
2	C	30	90	88	45
3	CS	25	80	86	46
4	CS	27	86	81	47
5	T1	34	79	88	46
6	T1	31	87	88	46
7	T2	29	82	64	42
8	T2	34	83	56	42

## S T A T I S T I C A L   A N A L Y S I S   S Y S T E M

## GENERAL LINEAR MODELS PROCEDURE

## CLASS LEVEL INFORMATION

CLASS	LEVELS	VALUES
TREAT	4	C CS T1 T2

NUMBER OF OBSERVATIONS IN DATA SET = 8

## S T A T I S T I C A L   A N A L Y S I S   S Y S T E M

(NOTE: C = Control; CS = Solvent Control; T1 = 0.77; T2 = 1.5)

## GENERAL LINEAR MODELS PROCEDURE

DEPENDENT VARIABLE: SUR

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F VALUE	PR > F	R-SQUARE	C.V.	
MODEL	3	57.37500000	19.12500000	1.46	0.3522	0.522184	4.2937	
ERROR	4	52.50000000	11.16666667		STD DEV		<sup>SUR-</sup> HAT MEAN	
CORRECTED TOTAL	7	109.87500000			3.62284419		84.37500000	
SOURCE	DF	TYPE I SS	F VALUE	PR > F	DF	TYPE IV SS	F VALUE	PR >
TREAT	3	57.37500000	1.46	0.3522	3	57.37500000	1.46	0.3522
S T A T I S T I C A L   A N A L Y S I S   S Y S T E M								4

7:55 Tuesday, December 21, 1982

## GENERAL LINEAR MODELS PROCEDURE

DUNCAN'S MULTIPLE RANGE TEST FOR VARIABLE: SUR

NOTE: THIS TEST CONTROLS ERROR RATES AT DIFFERENT LEVELS  
 DEPENDING ON THE NUMBER OF MEANS BETWEEN EACH PAIR  
 BEING COMPARED. ITS OPERATING CHARACTERISTICS SOMEWHAT  
 RESEMBLE FISHER'S UNPROTECTED LSD TEST.

ALPHA=0.05    DF=4    MSE=13.125

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

DUNCAN	GROUPING	MEAN	N	TREAT
	A	82.000	2	C
	A			
	A	83.000	2	CS
	A			
	A	83.000	2	T1
	A			
	A	82.500	2	T2

## GENERAL LINEAR MODELS PROCEDURE

DEPENDENT VARIABLE: LEN

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F VALUE	PR > F	R-SQUARE	C.V.	
MODEL	3	947.37500000	324.79166667	16.98	0.0097	0.927204	5.5445	
ERROR	4	76.50000000	19.12500000		STD DEV		LEN MEAN	
CORRECTED TOTAL	7	1050.87500000			4.37321392		78.87500000	
SOURCE	DF	TYPE I SS	F VALUE	PR > F	DF	TYPE IV SS	F VALUE	PR >
TREAT	3	974.37500000	16.98	0.0097	3	974.37500000	16.98	000097
S T A T I S T I C A L   A N A L Y S I S   S Y S T E M								10

7:55 Tuesday, December 21, 1982

## GENERAL LINEAR MODELS PROCEDURE

DUNCAN'S MULTIPLE RANGE TEST FOR VARIABLE: LEN

NOTE: THIS TEST CONTROLS ERROR RATES AT DIFFERENT LEVELS  
 DEPENDING ON THE NUMBER OF MEANS BETWEEN EACH PAIR  
 BEING COMPARED. ITS OPERATING CHARACTERISTICS SOMEWHAT  
 RESEMBLE FISHER'S UNPROTECTED LSD TEST.

ALPHA=0.05    DF=4    MSE=19.125

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

DUNCAN	GROUPING	MEAN	N	TREAT
	A	88.000	2	T1
	A			
	A	84.000	2	C
	A			
	A	83.500	2	CS
	B	60.000	2	T2

# GENERAL LINEAR MODELS PROCEDURE

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DEPENDENT VARIABLE: WT

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F VALUE	PR > F	R-SQUARE	C.V.	
MODEL	3	25.00000000	8.33333333	33.33	0.0027	0.961538	1.1111	
ERROR	4	1.00000000	0.25000000		STD DEV		WT MEAN	
CORRECTED TOTAL	7	26.00000000			0.50000000		45.00000000	
SOURCE	DF	TYPE I SS	F VALUE	PR > F	DF	TYPE IV SS	F VALUE	PR >
TREAT	3	25.00000000	33.33	0.0027	3	25.00000000	33.33	0.0027
S T A T I S T I C A L   A N A L Y S I S   S Y S T E M								13

7:55 Tuesday, December 21, 1982

## GENERAL LINEAR MODELS PROCEDURE

DUNCAN'S MULTIPLE RANGE TEST FOR VARIABLE: WT

NOTE: THIS TEST CONTROLS ERROR RATES AT DIFFERENT LEVELS

DEPENDING ON THE NUMBER OF MEANS BETWEEN EACH PAIR

BEING COMPARED. ITS OPERATING CHARACTERISTICS SOMEWHAT

RESEMBLE FISHER'S UNPROTECTED LSD TEST.

ALPHA=0.05   DF=4   MSE=0.25

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

DUNCAN	GROUPING	MEAN	N	TREAT
	A	45.500	2	CS
	A			
	A	46.000	2	T1
	A			
	A	45.500	2	C
	B	42.000	2	T2

## DISCUSSION/RESULTS

Based on the results of our statistical analysis we concur with the following statements made by the Testing laboratory.

"Percentage viability of rainbow trout embryos was unaffected by exposure to mean measured Norflurazon concentrations as high as 11.2 mg/l (Table 3). Percentage hatch of viable embryos exposed to mean measured concentrations of 11.2 and 5.5 mg/l Norflurazon was significantly reduced as compared to percentage hatch of viable control and solvent control embryos. Embryos began hatching on day 23 of exposure. None of the embryos exposed to 11.2 mg/l Norflurazon hatched successfully while newly hatched larvae exposed to 5.5 mg/l Norflurazon began dying after hatching. Mortality continued until day 9, post-hatch, at which time no surviving larvae remained in this treatment. Mortality and lethargic behavior were observed among larvae exposed to 3.3 mg/l Norflurazon on day 2, post hatch. These trends continued until day 17, post-hatch at which time no surviving larvae remained in this treatment. Lethargic behavior and mortality were observed among larvae exposed to 1.5 mg/l Norflurazon during the initial 15 days of exposure. Beyond day 15, post-hatch, surviving larvae no longer appeared lethargic and none died. Larvae survival was unaffected by exposure to a mean measured Norflurazon concentration of 0.77 mg/l. Mean total length and average wet weight of larvae exposed to 1.5 mg/l Norflurazon were significantly reduced as compared to control and solvent control larvae. Larvae exposed to 1.5 mg/l Norflurazon also appeared to have light pigmentation as compared to larvae in both control groups. Mean total length, average wet weight and pigmentation of larvae exposed to 0.77 mg/l Norflurazon was comparable to growth and pigmentation of control and solvent control larvae."

"Based on the reduced percentage survival and reduced growth of larvae exposed to a mean measured Norflurazon concentration of 1.5 mg/l, the MATC of this compound for the rainbow trout was estimated to be  $>0.77 <1.5$  mg/l."

## CONCLUSIONS

Category: Core